defective SNARE complex assembly and neurodegeneration (Sharma et al., 2011). It is thus conceivable that reactive intermediates produced during the SNARE cycle could induce a general age-dependent neurotoxicity (Mattson, 2003). Hsc70/Sgt form a trimeric complex with the synaptic vesicle-associated co-chaperone cysteine string protein α (Tobaben et al., 2001), and this complex uses the chaperone activity of Hsc70 to refold fusion incompetent SNAP-25 conformers (Sharma et al., 2011). Intriguingly, both Hsc70 and Sgt are upregulated with enhanced synaptic activity (Sharma et al., 2011). It is thus conceivable that changes in the relative levels of the two proteins in response to specific patterns of synaptic activity switch Hsc70 between its refolding chaperone function and its endosomal microautophagy function. As such, Hsc70 would switch from trying to refold SNAP-25 to targeting the protein, which has two microautophagy recognition motifs, to endosomal microautophagy.

Moreover, Uytterhoeven et al. (2015) find that ATP prevents oligomerization and the membrane deformation properties of Hsc70. Thus, in the presence of abundant ATP, Hsc70 would function as a chaperone but, with ATP deficiency, it would switch to a protein degradation mode. Remarkably, despite the fact that ATP synthesis is driven locally in the synapse and there is a large reservoir of ATP, the incredible metabolic demands of the synapse means that even brief interruption in activity-stimulated ATP synthesis impairs presynaptic function (Rangaraju et al., 2014). Thus, a simple switch in the local availability of ATP could provide an attractive mechanism to balance refolding versus degradation of synaptic proteins.

It is likely that synaptic endosomal microautophagy is but one of many processes that will be discovered to function in the local environment of the synapse to ensure the controlled activity of this key functional unit of the nervous system.

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Bridging the Gap: Muscarinic M4 Receptors Promote Striatal Plasticity in Health and Disease

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In this issue of *Neuron*, Shen et al. (2015) demonstrate that the M4 muscarinic receptor regulates striatal plasticity. The authors use an M4-positive allosteric modulator, which facilitates long-term depression in direct pathway neurons and reverses aberrant plasticity in levodopa-induced dyskinesia.

The basal ganglia's function in decision-making and action selection depends on robust plasticity, driven by sensory experience and feedback about action outcomes. Flexible behavior requires bidirectional plasticity: both potentiation and depotentiation (or depression) of synaptic strengths, which has been studied extensively in the input nucleus of the basal ganglia, the striatum. A fundamental unanswered question, however, is how corticostriatal long-term depression (LTD) occurs in striatal direct pathway neurons. While the mechanisms governing indirect pathway LTD have been studied extensively, delineating a parallel mechanism in direct pathway neurons has proved more difficult. In this issue of *Neuron*, Shen et al. (2015) close the gap in our knowledge of direct pathway LTD by demonstrating that type 4 muscarinic

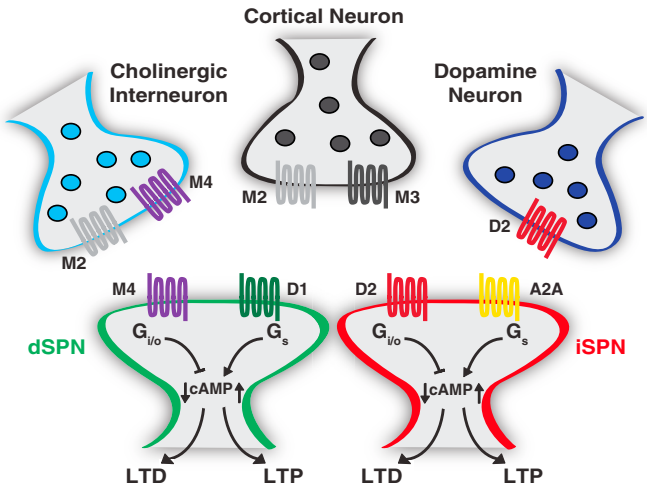

Figure 1. Parallel Pathways for Striatal Synaptic Plasticity
Major synaptic inputs (top) to striatal projection neurons (direct pathway, dSPNs; indirect pathway, iSPNs, bottom) are pictured. Key receptors and signaling molecules involved in synaptic plasticity are shown. Abbreviations: D2, dopamine type 2-like receptor; D1, dopamine type 1-like receptor; M2,3,4, muscarinic type 2,3,4 receptors; A2A, adenosine 2A receptor; cAMP (cyclic adenosine monophosphate).

receptor (M4R) activation facilitates direct pathway LTD. The authors apply this discovery to a neurological condition thought to arise from aberrant synaptic plasticity, levodopa-induced dyskinesia. This common complication of dopamine replacement therapy was reduced in two animal models of Parkinson's disease treated with an M4R positive allosteric modulator, uncovering a potential therapeutic target. Their findings support a long-hypothesized mechanism for direct pathway LTD and demonstrate the M4Rs may indeed be the missing link.

The basal ganglia are crucial for learning, using past experience to guide future decisions, which in turn affect motor planning and cognition (Yin and Knowlton, 2006). How is basal ganglia-dependent behavior shaped at the cellular level? Evidence supports bidirectional synaptic plasticity of excitatory inputs onto striatal principal neurons, or spiny projection neurons (SPNs), as critical both in learning and disease states (Kreitzer and Malenka, 2007; Yin and Knowlton, 2006). Rewarding outcomes regulate this plasticity through dopamine and other neuromodulators. SPNs receive glutamatergic inputs from the cortex and thalamus, local GABAergic and cholinergic inputs, and neuromodulatory input, including dopaminergic input from the midbrain (see Figure 1). SPNs can be subdivided into two intermingled groups, direct pathway neurons (dSPNs, which project directly to basal ganglia output) and indirect pathway neurons (iSPNs, which project via several synapses to basal ganglia output). Complementary sets of inhibitory and excitatory G protein-coupled receptors on SPNs are believed to regulate SPN long-term potentiation (LTP) and LTD. Direct pathway SPNs selectively express G_s-coupled dopamine type-1 receptors (D1R) and $G_{i/o}$-coupled type-4 muscarinic receptors (M4R). Conversely, indirect pathway SPNs selectively express $G_{i/o}$-coupled dopamine type 2 receptor (D2R) and G_s-coupled adenosine A2A receptors (A2AR). $G_{i/o}$ and G_s pathways bidirectionally modulate downstream signaling, including cAMP, regulator of G protein signaling type 4 (RGS4), and endocannabinoid release to control plasticity (Calabresi et al., 2000; Kreitzer and Malenka, 2007; Lerner and Kreitzer, 2012). Neuromodulators like dopamine, adenosine, and acetylcholine bias SPNs to LTP or LTD. Specifically, indirect pathway LTD depends on dopamine and D2R activation, as established by several groups (Calabresi et al., 1992; Kreitzer and Malenka, 2007; Shen et al., 2008).

How is direct pathway LTD regulated? The $G_{i/o}$-coupled M4R, located on dSPNs, has long been suspected based on its analogous intracellular signaling. M4R could act as a direct pathway analog of D2R, favoring LTD, whereas D1R could function in parallel to A2AR, favoring LTP. Like other striatal cholinergic receptors, M4R activation depends on local acetylcholine release by tonically active cholinergic interneurons. Multiple cholinergic receptor subtypes are expressed on many striatal cell types (see Figure 1), leading to a multiplicity of effects. Testing the role of M4R has been experimentally challenging for three primary reasons: (1) a lack of specific M4R agonists and antagonists; (2) cholinergic receptors on both pre- and postsynaptic elements, on almost every striatal cell type; and (3) the technical difficulty of inducing bidirectional synaptic plasticity with physiologically meaningful protocols in the slice preparation. Many plasticity protocols drive dopamine release (e.g., through local electrical stimulation); they may bias in favor of dSPN LTP and iSPN LTD, making it difficult to study dSPN LTD or iSPN LTP. Despite experimental limitations, several research groups have attempted to study M4Rs in healthy rodents and disease models (Gomeza et al., 1999; Martella et al., 2009). These findings, however, have not specifically addressed M4R in direct pathway LTD.

The development of highly selective M4 positive allosteric modulators (PAMs) (Shirey et al., 2008) is one promising approach. M4R PAMs do not activate the receptor directly, but interact with an allosteric site to increase affinity and coupling efficiency to G proteins (Shirey et al., 2008). Shen et al. (2015) used several novel M4R PAMs to identify how M4R signaling regulates striatal plasticity and levodopa-induced dyskinesia. This work builds on the group's prior work showing that dSPN spike timing-dependent plasticity (STDP) LTD could be induced in the presence of a D1R antagonist (Shen et al., 2008). However, Shen et al. (2015) combine STDP with pharmacology to show that M4R activation alone can facilitate LTD in dSPNs and reverse aberrant plasticity associated with levodopa-induced dyskinesia.

Several experiments by Shen et al. (2015) demonstrate that endogenous cholinergic signaling promotes direct pathway LTD. First, in ex vivo perforated patch recordings, they show the combination of the M4R PAM and a negative

timing STDP protocol induces direct pathway LTD, even without blocking D1R. Second, they show *postsynaptic* dSPN M4R (not M4R on other cells) are necessary for LTD; direct pathway LTD could not be induced in mice with cell-type-specific deletion of M4R in dSPNs, nor could it be restored with M4R PAM application. Third, when the authors artificially elevated cholinergic interneuron activity by cell-type-specific activation with the hM3D(q) DREADD, the same negative timing STDP led to robust direct pathway LTD. The authors went on to dissect downstream signaling, demonstrating that, like indirect pathway LTD, direct pathway LTD depends on RGS4 and endocannabinoids (Gerdeman et al., 2002; Lerner and Kreitzer, 2012), confirming a closely parallel process.

These findings provide support for the long-hypothesized role of the M4R in striatal plasticity, but also have behavioral consequences. Levodopa-induced dyskinesia is a common complication of long-term dopamine replacement therapy in Parkinson's disease. In this condition, therapeutic doses of the dopamine precursor levodopa trigger disabling involuntary movements. Presently, few therapeutic strategies exist to treat levodopa-induced dyskinesia. While the induction and expression mechanisms of levodopa-induced dyskinesia are still unknown, many hypothesize that dysregulated striatal dopamine and aberrant corticostriatal direct pathway plasticity contribute (Cenci and Konradi, 2010). Key evidence supporting this hypothesis includes alterations in glutamatergic synaptic function (Bagetta et al., 2012; Fieblinger et al., 2014) and loss of depotentiation in ex vivo slices from dyskinetic animals (Picconi et al., 2003). In vivo, excess direct pathway LTP may promote higher direct pathway activity during levodopa exposure, leading to involuntary movements. Preventing or reversing this aberrant plasticity might permit longer and more effective use of dopamine replacement therapy in Parkinson's disease.

To test the role of M4Rs in synaptic depotentiation, the authors used the M4R PAM in mice with levodopa-induced dyskinesia, produced by combining a common rodent model of parkinsonism with repeated levodopa treatment. To assess changes in striatal plasticity, they again used STDP, but used a positive timing protocol to trigger LTP. Consistent with prior findings, dSPN LTP was induced in levodopa-treated (dyskinetic) animals, whereas LTP could not be induced in untreated (parkinsonian) animals. Furthermore, the M4R PAM reversed the aberrant LTP associated with levodopa treatment. The authors tested whether this drug reduces levodopa-induced dyskinesia in vivo, administering levodopa and the M4R PAM to parkinsonian mice and non-human primates. Impressively, the M4R PAM lessened levodopa-induced dyskinesia in both models, and to a degree similar to an existing drug therapy, amantadine. This result implies that restoration of dSPN LTD/depotentiation can reduce levodopa-induced dyskinesia, and opens up additional areas of drug development.

While the results from this study expand our knowledge of M4R signaling and striatal plasticity, several questions remain. While the authors showed that dSPN M4R signaling was required to regulate LTD in vitro, in vivo the M4R PAM was given systemically; it may have multiple sites of action. Systemic administration is obviously more clinically viable, but to mechanistically connect M4Rs in direct pathway neurons, LTD, and a reduction in levodopa-induced dyskinesia, additional experiments would be required. M4R located on other cell types, including striatal cholinergic interneurons, may regulate plasticity; cell-type-specific modulation would help clarify this mechanism in vivo.

A major limitation of many candidate dyskinesia treatments is their predilection to reduce levodopa's therapeutic efficacy. For example, drugs which reduce dopamine release might reduce dyskinesia, but at the expense of antiparkinsonian benefit. The authors show some preliminary evidence that the therapeutic benefits of levodopa are not significantly altered with an M4R PAM, but this will need to be replicated in larger groups of animals with a wider range of doses. However, these findings represent a major contribution to our understanding of striatal plasticity, and are an impressive start in unraveling the clinical role of M4 PAMs in treatment of levodopa-induced dyskinesia.

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